2690-Pos Board B120
Molecular Dynamics Studies of the Ubiquitin Conjugation Mechanism
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Post-translational modification of proteins can have drastic effect on their structure and function. One such modification involves the attachment of a small protein, ubiquitin. An important function of ubiquitination is to signal proteins for cellular degradation. This process occurs in three enzymatic steps. In the second step, ubiquitin transfers to a conjugating enzyme, called E2, which then transfers ubiquitin to a lysine in the target protein. However, the mechanistic details for this final transfer remain obscured. Although it is clear that ubiquitin does bind, there are no studies that show exactly how this happens. The two most favored proposals involve a step-wise mechanism with a tetrahedral oxyanion intermediate and concerted mechanism. This work probes the accuracy of the oxyanion hypothesis. In particular, if the oxygen on the observed carbonyl carbon can form a stable hydrogen bond with the hydrogen on the nitrogen of the asparagine side chain, then oxyanion intermediate is plausible. By using molecular dynamics (MD), combined with umbrella sampling, a free energy profile of the formation of the breaking and forming of the hydrogen bond is constructed to see if its creation is thermodynamically favorable. Furthermore, information about the hydrogen-bonding environment in the active site is extracted.

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Proteolysis of Abnormal Prion Protein with a Thermostable Protease from a Hyper-Thermophilic Archaeon Thermococcus Kodakarensis Kod1
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The abnormal prion protein (PrPSc: scrapie-associated prion protein) is considered to be included in the group of infectious agents of transmissible spongiform encephalopathies. Since PrPSc is highly resistant to normal sterilization procedures, the decontamination of PrPSc is a significant public health issue. Tk-subtilisin is a subtilisin-like serine protease identified from a hyperthermophilic archaeon Thermococcus kodakarensis KOD1. Among the subtilisin family of proteases, Tk-subtilisin has significant high heat stability with its highest specific activity at 90°C and a half-life of 50 min at 100°C. In the present study, a hyper-thermostable protease, Tk-subtilisin, was used to degrade PrPSc. Although PrPSc is known to be resistant toward proteolytic enzymes, Tk-subtilisin was able to degrade PrPSc under extreme conditions. The level of PrPSc in brain homogenates was found to decrease significantly in vitro following Tk-subtilisin treatment at 100°C, whereas some protease resistant fractions remain after proteinase K treatment. Rather small amounts of Tk-subtilisin were required to degrade PrPSc at 100°C and pH 8.0. In addition, Tk-subtilisin was observed to degrade PrPSc in the presence of sodium dodecyl sulfate or other industrial surfactants. Although several proteases degrading PrPSc have been reported, practical decontamination procedures using enzymes are not available. This report aims to provide basic information for the practical use of a proteolytic enzyme for PrPSc degradation.

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Kinetic Characterization of Human Liver Phosphofructokinase
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Phosphofructokinase (PFK) catalyzes the phosphorylation of fructose 6-phosphate (F6P) to fructose 1,6-bisphosphate in an ATP dependent reaction. This reaction represents the first committed step of the glycolytic pathway and as such plays an important role in metabolism. In liver, this regulation is especially interesting, as hepatocytes can alternatively perform gluconeogenesis or glycolysis. While PFKs from the livers of several mammalian species have been characterized, human liver PFK has never been thoroughly examined. The gene encoding human liver PFK was synthesized and cloned into an expression vector utilizing the tac promoter. Human liver PFK was expressed in an Escherichia coli cell line, RL257, which contains no native PFK. The protein has been purified to a specific activity of 45 U/mg through a combination of ammonium sulfate precipitations, heat denaturation, and anion exchange chromatography. Initial characterizations indicate that human liver PFK is inhibited by ATP more substantially than rat liver PFK, a well characterized mammalian PFK. Additionally, pH changes modify the binding of F6P to a greater extent than in rat liver PFK. This work seeks to quantify the kinetic and allosteric behaviors of human liver PFK and contrast those behaviors with those of rat liver PFK. This work is supported by NIH grant GM033216.

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Use of 2-Photon Fluorescence Correlation Spectroscopy and Electron Microscopy to Ellucidate the Dependence of RLPFK Self Association on Ligand Concentration
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Phosphofructokinase (PFK) catalyzes the first committed step of glycolysis. Allosteric regulation of PFK makes its activity, and thus glycolysis, sensitive to intercellular metabolic conditions. Regulation of PFK from liver is complex because of the liver's responsibility to maintain glucose homeostasis, which means that glycolysis and gluconeogenesis must be regulated reciprocally. Allosteric regulation of PFK activity is achieved by modifying the binding affinity of its substrate, fructose 6-phosphate (Fru-6-P). However, in vitro studies performed on rat liver PFK (RLPFK) suggest that at physiological concentrations of Fru-6-P RLPFK activity is negligible even in the presence of known allosteric activators. Additional mechanisms must exist to account for the activity required to support flux through glycolysis. The smallest active oligomer of RLPFK is a tetramer, however fluorescence polarization studies have previously demonstrated that at a physiological enzyme concentration both Fru-6-P and other activators stabilize species much larger than a tetramer. A Weber linkage argument predicts that the highly associated species would demonstrate a higher affinity for Fru-6-P resulting in activation. RLPFK exists as a tetramer in the dilute enzyme concentrations necessary for in vitro activity assays whereas, at physiological concentrations, RLPFK can be highly associated. 2-photon fluorescence correlation spectroscopy (FCS) and electron microscopy have been used to quantify the oligomeric state of RLPFK at high and low enzyme concentrations. FCS performed on alexa-488 labeled RLPFK suggests a complex self-association behavior in the presence of Fru-6-P. Electron microscopy indicates that, in the presence of Fru-6-P, rat liver PFK can form long fibrils that consist of up to 36 PFK tetramers extending over 200 nm in length. These effects are counteracted by MgATP, a known allosteric inhibitor of the enzyme. Funding: NIH-GM33216, NIH-CBI and Welch-A1543

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The Isolated Large Subunit of E. coli Carbamoylphosphate Synthetase Deviates from Kinetic Mechanism and Allosteric Behavior of the Holoenzyme
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Carbamoyl Phosphate Synthetase (CPS) from E. coli is a heterodimeric enzyme translated from the CarA and CarB genes. The polypeptides formed from these transcripts are 42kDa and 118kDa, respectively. CPS catalyzes the synthesis of carbamoylphosphate through activation of bicarbonate by one equivalent of MgATP priming bicarbonate for the addition of ammonia; the carbamate is then phosphorylated by a second equivalent of MgATP. The product of the CarB gene contains two active sites, both are functional as partial reactions, one is responsible for bicarbonate activation by MgATP and the amination of the activated bicarbonate using an ammonia source (glutamine in vivo), the second site is responsible for phosphorylation of carbamate and is distal to the heterodimer interface. The binding site for allosteric regulators is located on the large subunit proximal to the phosphorylation active site. The smaller subunit contains the active site for deamination of glutamine and is not mandatory for the activity of the sites on the large subunit. Previous work demonstrated the reverse of the phosphorylation partial reaction of CPS, formation of ATP from MgADP and carbamoylphosphate, is equilibrium ordered and responsive to allosteric ligands. In this work we provide evidence that removing the small subunit, thus relieving interfacial constraints 45Å from the active site, results in reduced allosteric effects. Data shows that the kcat for the ATP synthesis reaction has decreased by 7-fold with no discernable difference in Kia. kcat = 6.9 sec-1 in holoenzyme and 1 sec-1 for the phosphorylation in isolated large subunit. The Kia determined for MgADP in holoenzyme is 510 μM and 700 μM in the isolated large subunit. Supported by the grant GM 33216 from the NIH.

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Free-Energy Landscapes of the Translocation of a Substrate in Four Proteasome - Activator Complexes Analyzed using Molecular Dynamics Simulations
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Proteasome is involved in the degradation of proteins. Proteasome activators bind to the proteasome core particle (CP) and facilitate opening a gate of the CP, where Tyr8 and Asp9 in the N-termini tails of the CP form the ordered open gate. In a double mutant (Tyr8Gly/Asp9Gly), the N-termini tails are disordered and the stabilized open-gate conformation cannot be formed. To